

Arbuscular Mycorrhizae for Sustainable Cul-Tivation of Grasses in Regenerative Agricul-Ture

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Abstract

This study focuses on solving rural problems, sustaining environmental conditions, improvement of pastures, and reduction of CO₂ concentration, suggesting inoculated vegetation types found in the tropics as a nature-based solution for degraded agroecosystems, besides for use as cover crops in regenerative agriculture. Noninoculated plants are intensively used for revegetation of degraded and mined lands, as well as for for arbuscular mycorrhizae (AMF) taxonomic and plant-fungal ecological studies. Among those grasses, *Urochloa brizantha* is a tropical forage, having vigorous deep roots, commonly used (AMF) multiplication in glasshouse and nursery, developing infective propagules to be conserved in germplasm banks. Other grass species, such as *Urochloa decumbens*, is also used for arbuscular mycorrhizal multiplication in addition to its cultivation for sustainable livestock systems. In the present study, a site of 100 m² was selected to take soil samples in a plain area, with *U. brizantha* pasture, and the soil chemical characterization, microcharcoal content and AMF spore identification, were evaluated 3 + 6 showing high amount of potassium (K), high microcharcoal content and the occurrence of 11.32 spores of AMF, most of Glomeraceae, in the rhizosphere of *U. brizantha*. Due to its economic importance for sustainable agricultural production and other uses besides its environmental services, as important promoters of soil carbon on grazing lands, more detailed research is needed on the biotic interactions and inoculant production of the studied grasses.

1. Introduction

Due to the increasing interest on grasslands for forage cultivation (source of meat and milk) to obtain a better food production besides reducing environmental unfavorable emissions of greenhouse gases, researchers look for decrease nutrient and fertilizers leaching and employ of pesticides, enhancing carbon sequestration, thus improving soil sustainability [1]. The deleterious effects of chemical inputs on the soil of monocultures are well studied [2]. Fortunately, the effect of manures, wastes, residues, compost, and biochar amendments on the soil system is increasingly studied worldwide, as organic agroecosystems are more procured. Thus, experiments on the implementation of natural soil conditioners by agriculture are rapidly increasing, and organic or regenerative agriculture adopted the addition of natural residues on horticultural plants and crops. Furthermore, the compatibility among biofertilizers and soil conditioners is also more investigated to support sustainable agricultural systems and to deal with the effects of global and especially, climatic change. Thus, the mycorrhizal symbiosis, application of compost from selected wastes [3], phosphate solubilizing microorganisms, microbial inoculants, and biochar, are increasingly explored. Of particular interest for research are perennial grassland plots of *Urochloa brizantha* (Fig. 1), a tropical forage (source of meat and milk), commonly used also for Arbuscular mycorrhizae fungi (AMF) multiplication, as this plant species associate with this type of fungi, which highly colonize its roots [4] developing infective propagules. Thus, *Urochloa decumbens* is also used for arbuscular mycorrhizae multiplication, in addition to its cultivation for ensuring the sustainability of livestock systems. Moreover, these grass species is increasingly used in no-tillage ecosystems as cover crops[5], such as in coffee trees [6]. Some grass species actively

propagate beneficial microsymbionts after their roots are colonized by the soil microbiota. This is the case of *Urochloa* (*Brachiaria*) species, which are largely cultivated in pastures and cover crops. *Urochloa* species are frequently selected for intercropping to improve land use and agricultural yields due to their high residue production, and slow decomposition [7]. The occurrence and life cycle of relevant symbionts such as the AMF, in grasslands were previously investigated, especially in Brazil [4]. Previous reports showed that *Urochloa* species are highly colonized by AMF, following the order: *U. decumbens* > *U. ruziziensis* > *U. humidicola* > *U. brizantha*. This grass is utilized in pest management and have supported scientific experiments (most involving comparisons between inoculated vs. non-inoculated plants regarding plant growth. Additionally, this grass is intensively used for revegetation of degraded and mined lands, AMF taxonomic studies and plant- fungi ecological studies. Related reports (various on AMF spore inoculation, and two influential works on AMF root colonization [4,8]. indicated differences between AMF species symbionts of *Urochloa* species. However, due to the economic importance of grass species, and the lack of complete data on this topic, more detailed research is needed to better understand the plant interactions with the soil microbiota. In the present study, with the purpose of examining the AMF associated to grasses in experimental farms, the AMF community structure was studied in a plot of *U. brizantha* grassland. Regarding spore numbers in the soil/ substrate, high values were previously observed in *U. brizantha* inoculated with *A. longula* (850 spores in 50 mL substrate), followed by plants inoculated with *Acaulospora colombiana* (755 spores) and *Acaulospora morrowiae* (228 spores, after 120 days of cultivation). In this sense, Barbosa et al. [8] highlighted the great multiplication potential of *A. longula* in *Urochloa*, thus indicating potential prolific species to be included as inoculants. In the present study, we reviewed, and examined the literature on the AMF associated to *Urochloa* species as well as the AMF-based inoculants in the field, including their benefits for plant cultivation. This paper examines the state of the art of *Urochloa* grasses in agroecosystems, with focus on its ecology including reports on their AMF symbioses. We searched the databases Scopus, and Google Scholar, with special attention to the most recent articles. In particular, the *U. brizantha* grassland plot at the experimental farm of EPAMIG was used for soil and fungal analyses.

2. Materials and methods

2.1. Study site

The *U. brizantha* grassland plot at the experimental farm of EPAMIG (19°42'51.5"S 44°53'37.0"W) in Brazil, South America, (estimated age of cultivation ~ 25 years, chopped up once a year, (Fig. 1) was used to study the AMF community structure. A site of 100 m² in the farm was selected to take soil samples in a plain area, with *U. brizantha* pasture, and the samples were collected between February (rainy period) and November (after the dry period) 2015.

2.2. Soil sampling and chemical characterization

Soil samples (three replicate plots (plot size, 5 by 20 m, composed by non-inoculated plants), obtained in February (rainy period) and November 2015 (after the dry period) at the site, were used for determination

of chemical soil parameters (Embrapa, 1997) and for AMF spore extraction. For physical and chemical soil analysis a composite sample from soil of each sampled point at each season was prepared. In total 9 soil samples from grassland ecosystem weighing about 0.5–1 kg each were transported to the laboratory using sealed plastic bags, For determination of chemical soil parameters (Embrapa, 1997).

2.3. AMF spore isolation and identification

AMF spores occurring in the soil samples (100g soil) were extracted by wet sieving, according to standard methods by Gerdemann and Nicolson [9], were counted and identified using specific descriptions of AMF species. The old and decaying spores with missing clear features were also counted as an approach for viability, according to Varga et al.[10], and the results expressed as percentage of unviable AMF spores.

2.4. Microcharcoal content

The number of microscopic (> 500 µm) charcoal fragments (adapted from[11], was measured on the same Petri dishes used for spore isolation at 400 × magnification using a grid, and results were expressed as number of fragments in the subsamples (25g soil).

3. Results

The *U. brizantha* grassland presented a Typic Acrustox soil (Soil taxonomy- USDA, 2022), (Table 2). The region's soil type was classified as sandy clay loam. The studied soil showed exclusive characteristics: high amount of K and higher charcoal content than samples of cultivated sites in the region (Table 3), studied previously by Pagano et al. [12]. The occurrence of AMF, most of Glomeraceae, was detected. Compared to other reports, *Claroideoglossum etunicatum* was dominant among the isolated species of Glomeromycota (Table 4). This study showed the AMF community associated to grasses, as well as potential for AMF inoculant formulation. Different AMF species (*Acaulospora longula* and *Acaulospora colombiana*) were previously reported by Barbosa et al. [8] as associated to *U. brizantha* in greenhouse, also in Minas Gerais, Brazil **Table 1** shows limited available reports on *Urochloa* species and their associated microbiomes in Brazil.

In the present study, the AMF community structure was studied in a grassland in Brazil, The AMF community structure in the *U. brizantha* experimental plot showed dominance of Glomeraceae, compared to other reports (Table 1) where *Acaulospora* and *Gigaspora* were inoculated and, thus, theoretically occurred, such as in the reports by Baptistella et al.[6, 7]. In the present work, five genera of Glomeromycota, four species and four unidentified morphotypes were detected in the *U. brizantha* grassland. The *U. brizantha* grassland showed exclusive characteristics: lower amount of K (Figure 2) and higher microcharcoal content (Table 2) than samples of cultivated sites in the region (Table 3).

Previous reports showed that The *Urochloa* species are highly colonized by AMF, following the order: *U. decumbens*> *U. ruziziensis* > *U. humidicola*> *U. brizantha* (Figure 5) (Adapted from dos Santos Lucas,et al., 2022).

This study showed the AMF communities associated to selected grasses at field conditions, as well as the potential for AMF inoculant formulation. Different AMF species (*Acaulospora longula* and *Acaulospora colombiana*) were previously associated with *U. brizantha* by Barbosa et al. [8]. (Table 1). However, to understand the *U. brizantha* ecology it is necessary to analyze the associated AMF species, the time of symbiosis establishment, root colonization and spore multiplication under different abiotic and biotic conditions. The hypothesis of this study is that *U. brizantha* associate with diverse AMF species, which have different behaviors when colonizing this host plant. We examined the arbuscular mycorrhizae associated to this grass in an experimental farm. The community structure was studied in a *U. brizantha* grassland in Brazil (Figure 3a), which showed a greater diversity of AMF compared to existing previous reports. Moreover, about 50 % of unviable AMF spores was estimated. Four genera of Glomeromycota, four species and four unidentified morphotypes were detected (Table 3). The grass showed exclusive characteristics: higher amount of soil organic matter and higher macroaggregates and charcoal content than samples of cultivated sites investigated in the region by Pagano et. al. [12] Most studies were carried out in glasshouse (pot experiments); however, the present study is the first to show in detail the AMF community associated to *U. brizantha* experimental plot (field site) besides previous reports on root colonization [4,12], and to point out the potential for AMF inoculant formulation to improve sustainable grass cultivation at field conditions.

AMF spore number

In the present study, a mean of 1.32 spores of AMF, most of Glomeraceae, were retrieved in the rhizosphere of *U. brizantha*

Table 4. AMF species associated with *Urochloa brizantha* at the experimental plot in the farm, Minas Gerais, Brazil.

In the report by Kanno et al.[13]; mycorrhizal and soil pH treatments significantly affected shoot, root, and total dry weights of the tested grass species. Though inoculation with AMF increased plant growth, the responses varied among the plant species (*U. humidicola* did not responded to inoculation). Shoot, root, and total dry plant weight increased as pH increased; however, between the mycorrhizal treatment and soil pH, there was a significant interaction for root dry weight and total dry weight. AMF increased root dry weight significantly only at low pH, and total dry weight only in the low and high pH treatments. In previous studies, roots were highly colonized when inoculated, compared to than non-inoculated ones [8] (Table 5).

4. Discussion

Most previous reports on *Urochloa* come from greenhouse experiments, indicating differences between AMF species associated to *U. brizantha*, *A. colombiana*, showing the greatest percents of AMF root colonization in a short cultivation time, as evaluated by (Barbosa et al.[8] who found the highest number of AMF spores of *Acaulospora longula* and *A. colombiana*, exhibiting the highest mycorrhizal root colonization after 76 days, confirming that the inoculation favored the root growth of *U. brizantha* in

greenhouse. The need of more information for a better understanding of the symbiotic relationships between different AMF species associated to *U. brizantha* was earlier pointed out and thus, we hypothesized that this plant species associates with a diverse community of AMF, including Acaulosporaceae, Gigasporaceae and Paraglomeraceae representants, as found in the AMF inoculation experiments and reports mentioned above. However, more investigation is needed regarding effectiveness of indigenous inocula and to compare it with commercial inocula formulation. In the present field study, the results indicated at least four AMF species (Fig. 3), which can be propagated on farm, establishing sustainable *Urochloa* cultures in tropical agroecosystems. Also, *Sorghum* cultures (Fig. 2b) cultivated in pots with *U. brizantha* grassland soils can be employed. As AMF species vary in their performances for the different plant species, it is needed to compile the knowledge based on these grasses to optimize their management. In the present study, the AMF species were most from Glomeraceae, which are designated as r-strategists, and are commonly found in anthropized agroecosystems; however, some studies showed the effective potential of *Acaulospora*. Thus, it is necessary to study the different AMF species (such as the great variety of *Acaulospora*) and propagules during the cultivation time to better understand the symbiosis in *Urochloa* species and other promising tropical forages. The production of biological inputs on farm (rural properties) is also increasingly implemented. The production of biological inputs such as inoculants and products for biological pest management is guided by the Ministry of Agriculture, Brazilian Agropecuary Company (EMBRAPA) for monitoring and regulate the quality and safety of the tested products.

4.1. *Urochloa* species for cover crops.

Perennial grasses have been increasingly employed as cover crops, and intercrops, besides their use for forage, and they were also utilized in pest management and for supporting scientific experiments. Among the *Urochloa* species, *U. humidicola*, *U. decumbens* and *U. ruziziensis* were investigated [4], presenting percents of AMF root colonization of 55, 78, and 66%, respectively. These high values indicate the relevant role of these plant species benefiting soil health through the fungal network provided by AMF colonizing the roots, which benefits plants and protect the soil. Furthermore, AMF secrete a protein (glomalin), which favors soil aggregation, improving soil quality. In this sense, only one report showed the soil glomalin content in *U. brizantha*, which, varied from 3.30 to 3.74 mg. g soil⁻¹ when inoculated with *G. margarita* and *A. longula*, respectively [6]. The low glomalin content when inoculated with *Gigaspora* sp. is expected as this AMF species grows slowly, compared to other ones, presenting a characteristic K-strategy lifestyle. Due to the relevant role of cover crops and intercrops in increasing diversity and sustainability of crops, they are commonly used by several reasons: the residues left after cutting protect the soil and provide nutrients to the next crop or intercropped culture. Additionally, *Urochloa* species have vigorous, abundant deep roots, faced to the superficial and scarce roots of crops. These grass traits facilitate carbon sequestration, soil organic matter stabilization and nutrient cycling. *Urochloa* roots also improve soil physical characteristics and influence soil nutrient dynamics, reducing nutrient losses and enhancing their cycling, which is benefic for nutrient use efficiency in sustainable agriculture. Furthermore, these cover crops are commonly employed in coffee trees [6] and in many

orchards. Thus, meliorating the *U. brizantha* culture via benefits from AMF and increasing research on the symbionts and related microbiota, will favor sustainable grassy ecosystems destined to forage, which can also provide sustainable plant systems to reproduce AMF propagules. Therefore, *U. brizantha* has showed high root colonization (35%) when inoculated with *A. longula*; however, low values (16%) were reported when inoculated with other species, such as *G. margarita* (16%) or *Paraglomus occultum* (27%). The low root colonization when inoculated with *G. margarita* may be related to the characteristic slow root colonizer of this AMF species. The cultivation of *Urochloa* in agroecosystems have multiple benefits, such as reduced risk of soil erosion, better soil structure, higher levels of soil organic matter and biological activity [7].

4.2. *Urochloa* species for AMF studies

Urochloa brizantha is a tropical forage, having vigorous deep roots, commonly used for arbuscular mycorrhizae (AMF) multiplication (AMF-multiplier plant) in glasshouse and nursery, developing infective propagules for conservation in germplasm banks and, thus, to be used in taxonomy studies of AMF, providing information on the interactions of different AMF for mycorrhizal colonization and spore multiplication to be used in AMF collections and experiments. At the same time, most propagules provide information on the interactions of different AMF for mycorrhizal colonization and spore multiplication to be used in AMF collections. As AMF species used in the studies are identified by a deposit code in the AMF collection of each University or Research Institute, in Brazil. These AMF have unique value for inoculum to be applied in experimental research or in farms. Other grass species, such as *U. decumbens*, is also used for arbuscular mycorrhizae multiplication. Traditionally, a mix of dominant AMF species recruited from the local site can be employed. However, it was common to produce inoculum combining two different AMF species (*G. margarita* and *Glomus* sp.). In other studies in greenhouse, Pedroso et al.[14] applied a mixture of spores (*Glomus macrocarpum*, *P. occultum* and *Glomus* sp. to inoculate *U. brizantha* in soil contaminated with heavy metals. They found greater root colonization in inoculated plants, showing beneficial effects of AMF on plant growth and alleviation of soil contaminants. As obligatory biotrophic, the multiplication of AMF is completed by axenic in vitro cultivation using transformed roots, or by its classical form, in cultivated trap plants according to Selvakumar et al. [15]. In vitro cultivation can enable the multiplication of cleaned (non-contaminated) spores in a shorter interval, being applicable for multiplication of fast-growing AMF species, such as those of Glomeraceae (mainly *Glomus intraradices*). It is also common to establish this symbiosis using *G. margarita*, isolated from introduced or autochthonous *G. margarita* isolates. For instance, AMF inoculum of *G. margarita* can be produced against species deposited in Glomeromycotan Germoplasm Banks, at the Universities, Institutes, or also in farms, isolated from introduced or autochthonous *G. margarita* spores, which can be multiplied in plastic vats containing ~ 20 kg of substrate(generally, a mixture of soil and sand 1:1 (v:v), having *U. decumbens* as host plant is utilized. During the period of growth, the plants are fertilized with nutritive solution (50% P), but then, only water is used. Five months after planting, for drying the plants and substrate, irrigation is finished. Subsequently, the substrate is removed and sieved, and roots removed from soil, cut into fragments, as inoculum of more effective species for plants of economic interest. Thus, the knowledge of interactions between macro and

microsymbionts, especially those positive, contributes to the introduction and targeted management of fungi in agroecosystems, selecting populations of more effective species for plants of economic interest. A great number of studies have utilized AMF spores multiplied using *U. decumbens* for propagation of spores used as inoculum.. Early reports by Kanno et al. [13] showed indigenous arbuscular mycorrhizas for 1 L inoculum suspensions, obtained against pasture soils, containing *Gigaspora* spp., *Scutellospora* spp., *Glomus* spp., *Acaulospora* spp. and *Entrophospora* spp., which was formulated based on microscopic observation. The grasses tested were *Brachiaria brizantha* cv. Marandu, *B. decumbens* cv. Basilisk, *B. humidicola* cv. Humidicola and *Panicum maximum* cv. Tanzania. That inoculum was used to inoculate the grasses in a glasshouse pot experiment where the percentage of root colonization by AMF per pot for each grass species and soil pH treatment (High, medium, low) was estimated. Average percentage of root colonization by AMF varied between 33% and 52% in the inoculated treatments (Table 4), contrasting to the uninoculated plants, which presented less percent (5.6 – 15.6%) of roots colonized by AMF. To compare the advantages of non-AMF- and AMF-inoculated plants is a common practice for inoculum production. In this sense, most studies testing inoculants were achieved in greenhouse, microcosm, or nursery, employing sterilized soils or substrates. Among the reported AMF species belonging to the genus *Gigaspora*, *G. margarita* was initially one of the most utilized species, maybe due to its easily to distinguish characteristics and great spore size. Therefore, there are commonly used species, which can be included in farm inoculation of native AMF at field to improve grass cultivation.

Regarding pH values for soil treatments, early reports firstly stated that AMF improved the growth and P uptake of *U. decumbens* at low and high pH levels, but not at an intermediate level. (5.7), concluding thus that the significance of AMF differs with values of soil pH and not only with the grass species.

4.3. *Urochloa* species for carbon mitigation

Besides the contribution of *U. brizantha* plants fixing atmospheric carbon dioxide CO₂, the accumulation of glomalin (a glycoprotein produced by AMF hyphae and spores) in the soil is important due to its role protecting organic carbon, which contributes to the reduction of CO₂ emissions. Thus, improving the growth of *Urochloa* plants results in a greater photosynthetic activity (increased fixation of atmospheric CO₂, favoring the accumulation of organic carbon by roots and increasing the nutrient cycling in the soil/plant system. Glomalin, a glycoprotein found in the soil, produced on the surface the extra-radical hyphae (mycelium), intra-radical hyphae on roots in the rhizosphere, and by degradation of the AMF hyphae and spores, constitutes a stable form of carbon storage in the soil, according to Rillig and Lehmann et al. [17,18]. Thus, higher AMF hyphal length increases glomalin content, which contains 30–40% stable C, persistent in soils (as evaluated by Hossain et al.[19]. In this sense, it is known that low glomalin content (0.007 mg per gram soil) was reported for Antarctic region, and higher contents (13.50 mg per gram soil) were common in tropical rainforest. On the other hand, in agricultural soils, glomalin content can vary between 0.30 and 0.70 favoring the accumulation of organic carbon by roots and increasing the nutrients flow and cycling in the plant-soil system. Therefore, The AMF increase plant growth, foliar biomass, and photosynthetic rate, and thus, fixation of atmospheric CO₂), favoring the

accumulation of organic carbon by roots, and the nutrient flow in the plant-soil system, as pointed by Wang et al. [20] and Lehmann et al. [18]. Furthermore, regarding carbon mitigation potential of tropical croplands, it is known that grazing lands occupy ~ 3.6 billion ha, accounting for one-fourth of possible C sequestration of worldwide soils. Based on global estimates, they can remove ~ 20% of the CO₂ released annually into the earth's atmosphere from global deforestation and land-use changes. Atmospheric CO₂ is incorporated by grazing lands through photosynthetic assimilation by grasses, and C sequestration by way of soil organic carbon (SOC). Therefore, grazing lands provide environmental benefits besides their economic returns. Among the environmental benefits provided by grasses, the maintenance of surrounding soil and water resources, air quality, and habitat for human and wildlife are mentioned. Thus, grasslands contribute to the economic well-being, exchange of products derived from them at local, regional, or national scales. Rates of SOC sequestration vary with climate, soil, and management. In this sense, several examples have showed the SOC sequestration attained by public policy efforts, as informed by Follett and Reed [21]. Additionally, it is known that 80% of the Total C saved in terrestrial ecosystems (approximately 3170 gigatons) is found in soils [22]. Soil respiration is one of the largest C fluxes from terrestrial ecosystems to atmosphere, as pointed by Luo and Zhou[23], minor changes in soil respiration can affect the C budget of soil. (as reported by Zhao et al. [24]. Soil carbon sequestration (process in which CO₂ is removed from the atmosphere and stored in the soil, primarily mediated by plants, with carbon stored in the form of SOC, as pointed by (Lal[22] and (Ontl et al. [25]. Moreover, the tropics often have intermediate SOC levels due to high rates of primary productivity and decomposition due to the hot climate and profuse precipitations. It is known that promoting soil C management on grazing lands is recommended for reduction of emissions in compensation emission strategies. Soil carbon management has multiple societal benefits, including greenhouse gases (GHG) emission reduction in climate change mitigation programs. It is also known that grazing can affect the root C allocation between the synthesis of new tissue and the C loss caused by root exudation and respiration, as pointed by Wilson et al.[26]. Grazing changes the physicochemical properties of soil (SOC mineralization, composition, and activity of microbial communities, according to Qu et al. [27], and by this means affects the C dynamics of soils, as verified by Hafner et al. [28] due to manure and urine released by livestock, as related by Vandandorj et al. [29].

4.4. *Urochloa* species for forage

this perennial monocotyledon is the principal source of diet for ruminant cattle in tropical and subtropical areas worldwide, such as the Americas, Africa, but especially in some countries of South America, such as Brazil, where animals are raised on pastures established with four significant *Urochloa* species (*Urochloa brizantha*, *Urochloa decumbens*, *Urochloa humidicola*, and *Urochloa ruziziensis*). Due to their economic relevance, these plant species were deeply studied, as Brazilian livestock production is concentrated in natural pastures» degraded planted pastures» pastures planted in good conditions[30,31]. Regarding plant differentiation, there were recognized two complexes of productive forages: 1)the *brizantha* complex (*U. brizantha*, *U. decumbens* and *U. ruziziensis*), and 2) the *humidicola* complex (*U. humidicola*, *Urochloa dictyoneura*. Also, *Urochloa maxima* was previously transferred to *Megathyrsus* and *Panicum*, as reviewed by Tomaszewska [32]. Among tropical forages, *Urochloa* and

Megathyrsus (syn. *Panicum*) present production potential in the tropical region of Brazil., due to their facility to adapt to different soil types and soil fertility, besides growing at different climatic situations, thus, *Urochloa* is one of the most planted grasses in Brazil, which livestock production is based on the use of pastures, (~ 158,622,704 hectares, (46,847,430 hectares of natural pastures, 11,821,006 of degraded planted pastures, and 99,954,268 hectares of pasture planted in great condition). Pastures of *U. brizantha* cv. Marandu are the most utilized forage in Brazil, responsible for ~ 40% of the sales of forage seeds cultivated in more than 70 million hectares [31], as verified by da Silva. [20], The *Cerrado*, a Brazilian savanna, which covers ~ 2 M km² in the center of the country, is an important area for livestock production in the world. Since the 1970s, an estimated 0.5 M km² has been occupied by tropical grasses of African origin such as *Urochloa (Brachiaria)* or *Panicum maximum* [32]. However, since the soils of the Brazilian savannas are highly acid, and low in phosphorus (P), the productivity and sustainability of the improved pastures are mainly affected by the ability of the established grass to extract P from the soil [33].

4.5. *Urochloa* for revegetation

Urochloa is commonly used in revegetation and reclamation of degraded, contaminated, and mined soils. Early works have showed that revegetated mined soils using *Brachiaria (Urochloa)*, and N₂ fixing plants such as *Mucuna* and *Crotalaria*, commonly present in the seed cocktails used by mining companies, was a frequent approach. In riparian zones, Novais et al. [34]. used the practice of spread out a mixture of seeds rich in nitrogen-fixing plant species with sand and composted cotton, resulting in a rapid establishment of native plants plot. like the control reference site (Cerrado vegetation) thus, fortunately, *U. brizantha* was not dominant, and the low soil organic carbon indicated low plant diversity, showing that the competition caused by *U. brizantha* can affect the ecological diversity in areas experiencing ecological succession. *Urochloa humidicola*, *Panicum maximum*, and *U. brizantha* were tested in an experiment carried out throughout more than 10 years in a coal mining area in Candiota, Southern Brazil. Those species were compared to natural soil under native vegetation, which is considered a positive control or reference by Miguel et al. [35]. After that time those grasses improved soil physical conditions up to 10 cm depth. Moreover, as these sites are impacted by machine traffic events in the topsoil layer, they found an increase in the percentage of soil macroaggregates from zero traffic decreasing to twelve events of movement. Also, simultaneous reduction in the percentage of soil microaggregates was observed. In Previous studies of revegetation with perennial grasses also in Brazil (8.6-years) roots enhanced minesoil structure at 10 cm depth disrupting compacted aggregates caused by compression of dense machinery traffic, as pointed by Stumpf et al., 2016) [36]. Several works conducted for revegetation employed seeds of rough, aggressive *U. decumbens* and *Melinis minutiflora* for creation of a creeping layer, after soil correction and recover rapidly the desired areas. The seeds used to form the “cocktail of seeds” can be collected in adjacent areas of National Parks or used commercial ones. Sowing can be done manually, and the seeds can be disposed at a depth of 2 cm. *Urochloa* has been used especially in revegetation slopes of highways, as showed by Santos et al. [37].

4.6. Fertilization and Soil amendments for *Urochloa*

4.6.1. Fertilization

Urochloa species can contain $\sim 100 \text{ kg ha}^{-1}$ of N, 130 kg ha^{-1} of K, 15 kg ha^{-1} of P, 40 kg ha^{-1} of Ca and 25 kg ha^{-1} of Mg, as pointed by Baptistella et al. [7]. Once correcting nutrient deficiencies, nitrogen availability controls plant production through assimilation of CO_2 for growth. Among pastures of *Urochloa brizantha* cv. Marandu, maintained at a height of 25 cm, it was also shown that weight gain per animal was similar between different doses of nitrogen(180 kg/ha of 4-14-8 fertilizer (urea, P_2O_5 , and K_2O) applied to grass, as reported by Barbero et al. [38]. Moreover, Kanno et al. [13]. added 54.4 mg/kg urea (24.5 mg/kg N), as a basic fertilizer in pot experiments seemed with *Brachiaria brizantha*. It is known that the substitution and removal of nutrients significantly affects the soil and plant conservation, and, thus, the conservation of pastures, therefore, soil fertilization is often carried out. Oliveira et al., (2024) tested wood ash waste as an organic fertilizer in cultivated *Urochloa brizantha* in the Cerrado, the great Brazilian pastureland, showing that the application of wood ash, in selected doses (16 to 24 t ha^{-1}) can be used as soil conditioner and fertilizer in pastures. *Urochloa* is thus potentially benefic for sustainable agricultural production as well as pasture.

4.6.2. Soil amendments

Regarding soil amendments, biochar-based fertilizers produced against poultry litter promoted 11% higher biomass production in *U. brizantha* cultivated pots under greenhouse conditions, as revealed by Lustosa Filho et al.[39]. Biochar is a promising material for improving plant and AMF performances, as it is insoluble in water and presents low short-term availability (pointed by those authors). However, it can increase over time due to the fertilizer dissolution when water distributes into the biochar pores, and at the same time, it can profit from discarded biomass promoting sustainable agriculture.

5. Genetics of Urochloa species

Regarding the genetic structure of these plant species, there is great potential for more productive cultivars with high nutritional quality, resistance to pests and diseases, and tolerant to abiotic stresses more efficiently, and sustainably available in the future, as proposed by Ferreira et al. [40] However, the genetics and genomics of *Urochloa* pastures has been few investigated, existing a research gap, mainly due to their genomic complexity. [40], limited breeding programs, and few sequenced genomes of diploid *Urochloa* species currently available.

5.1. Inoculation of Urochloa species

Rhizophagus (46%), *Funneliformis* (41%), *Claroideoglomus* (15%), *Glomus* (9%) and *Gigaspora* (2%) were frequently obtained in commercial inoculants. In this sense, Basiru and Hijri, [41] recently reviewed the application of commercial AMF inoculants and the ecological effects on the indigenous fungal communities under field conditions, highlighting the need to focus on selected species with a higher chance of success in the field, having minor side impacts. However, It is known that AMF commercial

inoculants contain a few *Glomeraceae* species (*Rhizophagus*, *Funneliformis*, and *Claroideoglossum*) and that inoculants consisting of several AMF species can produce additive or synergistic effects on target crops. In the first studies, *U. decumbens* was inoculated with *G. margarita* and *Glomus. clarum* to study the recovering of degraded lands in greenhouse experiments, as showed by Santos et al.[42]. For mycorrhizal treatments, there are various possible procedures: 1) small holes can be prepared in the soil/ substrate surface of each pot, and the inoculum (~ 2.5 mL) placed in each hole: 2) Other procedure is to throw the spores into the roots of each plant(before transplanted) to pots. It is also more recently utilized to add (In the non-mycorrhizal treatments), 1,25 mL of the filtered leachate in each hole, to avoid the differences due to other microorganisms(bacteria) living together with the fungal components; however it may cause contamination. Moreover, it was recently showed by Vather et al.[43]. that commercial AMF inoculants do not contain the species they declare. Thus, more research is needed on AMF inoculants at field conditions and their impacts on native communities, establishment in soil and roots, persistence, and influences on the native AMF community. In this sense, most studies indicate that introduced AMF can stay in the target field only a few months or some years decreasing abundance (60%) or with exclusion (30%). Further analysis shows that AMF inoculation exerts both positive and negative impacts on native AMF species, including suppression (33%), stimulation (38%), exclusion (19%), or neutral impacts (10% of examined cases). The factors influencing the ecology of AMF inoculants, such as the inherent properties of the inoculum, dosage, and frequency of inoculation, besides soil physical and biological factors, were discussed by Basiru and Hijri, 2022) [41]. In a different approach (Fertigation), Karti et al. [44] tested the inoculum of AMF acquired by hydroponic procedure, (spray, drip, and Nutrient Film in *U. decumbens* with different nutrients, as AMF promote adequate nutrition in soils.AMF colonization is present in *U. ruziziensis*>

6. Conclusions

This study has detailed the AM fungal community associated with *Urochloa* roots in a tropical grassland ecosystem using morphological methods for AM spore identification. Moreover, we compiled available reports on this plant species and its cultivation in tropical South America, expanding the topic with mention of major constrains and benefits of its cultivation and gaps to be solved in the future. *Glomeraceae* was the most prolific fungal family inhabiting the grassland; however, other AM fungal species were reported associated to this host along the period of more than twenty years. We confirmed four AMF species and four morphotypes associated to *U. brizantha* in a tropical managed soil. This study contributes for the selection of highly-efficient inoculants for the mycorrhizal colonization of plant roots, especially, *Urochloa* species (*U. brizantha*), at field conditions. For instance, *F. geosporus*, *D. heterogama* and *C. etunicatum* can be indicated as potential inoculants for *U. brizantha* plots in this tropical region. Based on the findings of Vather et al.[43] who revealed few AMF species present in commercial inoculants., thus, the present study is valuable for grass cultivation, pointing out the need for well examining the AMF composition for a more precise inoculum available to the farmers and to the market, based on the evaluation of inoculum of AMF in *Brachiaria decumbens* [44]. As reviewed by Baptistella [7], it was highlighted that inoculation preferentially of *Acaulospora* species, favors the root

growth, plant height, root and shoot dry matter of *U. brizantha* in tropical agroecosystems. However, in the present study, *Claroideoglomus* and *Dentiscutata* species are also indicated to be tested as inoculants. Thus, we recommend more detailed and long-term studies on the associated AMF species and their benefits for soil aggregation, as well as evaluation of glomalin content in more samples of *Urochloa* soils. Earlier investigation showed that the *Urochloa* species are highly colonized by AMF, following the order: *U. decumbens* > *U. ruziziensis* > *U. humidicola* > *U. brizantha*, thus, we need more information on the microorganisms associated to these plant species.

Declarations

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Ethics approval and consent to participate: All authors approve and participate in the manuscript

Consent for publication: All authors approve the publication of the manuscript

Availability of data and material: Data available as requested.

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Tables

Tables 1 to 5 are available in the Supplementary Files section.

Figures



Figure 1

grassland plot of *Urochloa brizantha* at the experimental farm.

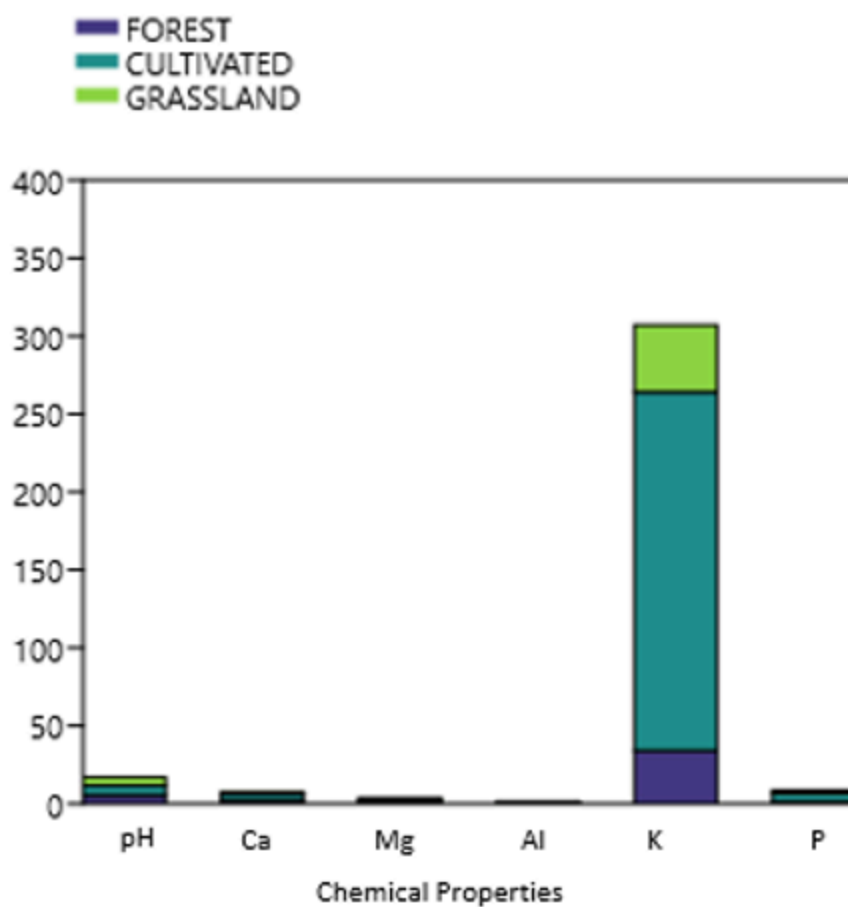


Figure 2

Soil elemental composition at different ecosystems in Pitangui experimental farm, grassland, cultivated site and adjacent forest.



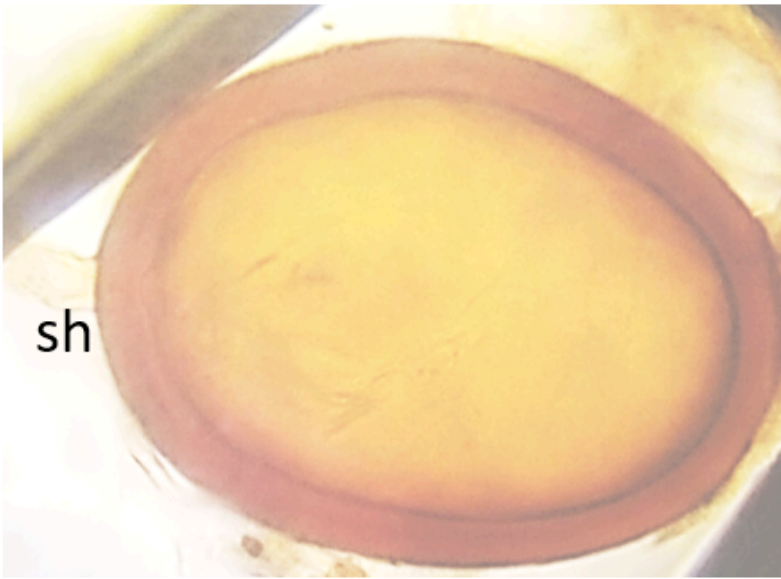
(A)



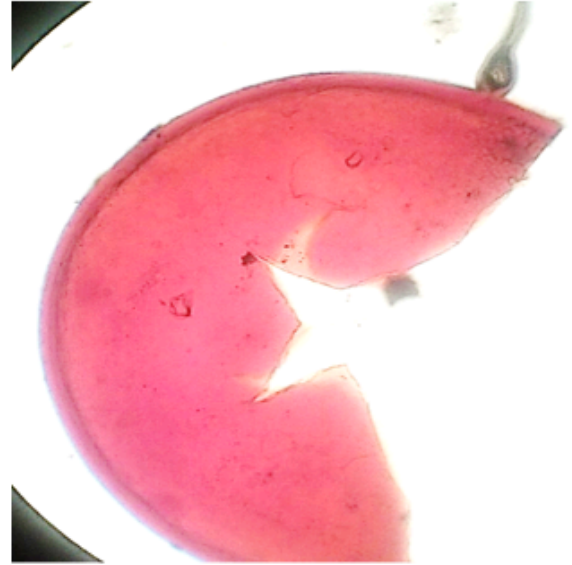
(B)

Figure 3

U. brizantha grassland in Brazil, (A). *Sorghum* cultivated in pots (B) with *U. brizantha* soil for AMF multiplication.



(A)



(B)

Figure 4

AMF species found in the U. *brizantha* grassland. *Claroideoglomus etunicatum*, with hyaline subtending hyphae(sh) (A), *Dentiscutata heterogama* (B) with sporogenous cell (sc).

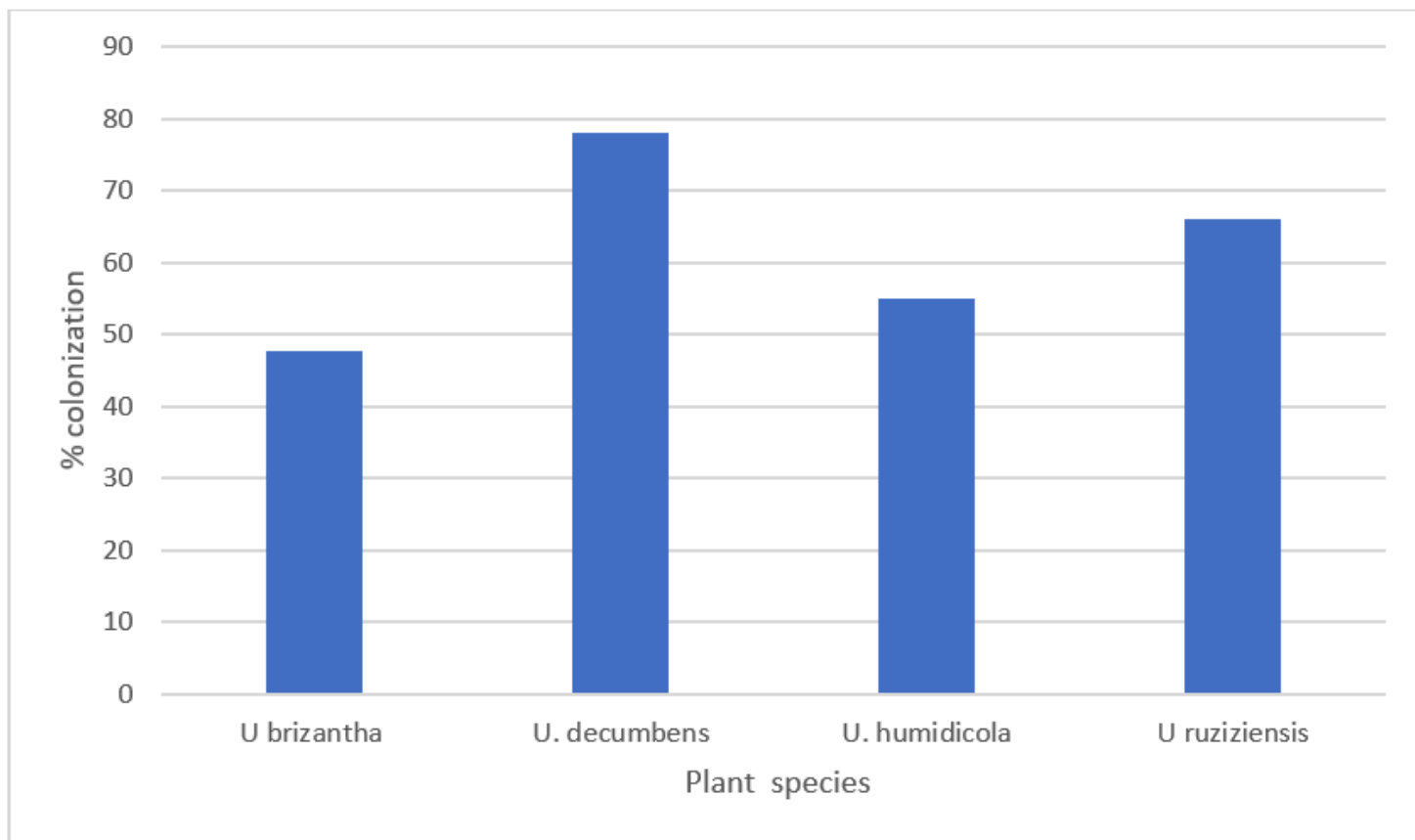


Figure 5

AM root colonization(%) in different *Urochloa* species. Adapted from dos Santos Lucas, et al., 2022

Supplementary Files

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